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The objective of this study was to identify differences in expressed genes in algae that live as symbionts in sea anemones. We compared several characteristics of algae as they lived in host tissue with those of the same algae when grown in culture outside of the host. The cultured algae exhibited none of the carbon release that is characteristic of symbionts living in the host. However, if the cultured algae were grown in host tissue, they developed normal release patterns within two months. Nutrient supply for the algae may have been responsible for these differences. Phosphatase enzyme patterns also differed between the cultured and the symbiotic states, and these appeared to reflect differences in pH environment. Cultured symbionts were much less infective in host tissue than freshly isolated symbionts. Ultrastructural evidence indicated that this was related to differences in cell surface structure.

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FINAL REPORT

GRANT #: N00014-96-1-0602

PRINCIPAL INVESTIGATOR(S): Dr. Clayton B. Cook

INSTITUTION: Harbor Branch Oceanographic Institution

GRANT TITLE: "Genetic Regulation in the *Aiptasia pallida* Symbiosis"

AWARD PERIOD: 1 March 1996 - 28 Feb 1999

OBJECTIVE: To understand gene regulation in the symbiosis between dinoflagellate symbionts (zooxanthellae) and their sea anemone host, *Aiptasia pallida*.

APPROACH: We used two general approaches in addressing this question. In one approach we prepared samples of the dinoflagellate symbionts (*Symbiodinium bermudense*) as they occurred in host tissue and in culture. The samples were sent to The Institute of Genomic Research (TIGR), so that cDNA libraries could be constructed from each. Comparisons of expressed sequence tags (EST's) from these libraries would have indicated genes that were expressed under the two different conditions.

In the second approach, we examined various physiological differences between the symbionts in culture and in the intact symbiosis, with the hope that some of these phenotypic differences would correspond to differences in the EST's in the TIGR study. We focused on three areas: (1), the release of photosynthetically fixed carbon by the symbionts, and its use by the host; (2), the expression of phosphatase enzymes by the cultured and symbiotic algae, and (3), the role of the cell surface in mediating recognition during the establishment of the symbiosis.

ACCOMPLISHMENTS: The DNA work with TIGR proved unproductive. At Harbor Branch we developed methods of isolating the native symbionts with little or no contaminating host cell DNA. Although we provided TIGR with cells in both the symbiotic and cultured states, no cDNA libraries were constructed. This was in part due to the departure of the two PI's involved (Drs. L. FitzGerald and Dr. H. Tomb), both of whom left TIGR for other positions during this project.

Release of photosynthetic carbon by the symbionts. The release of photosynthetic carbon to host tissue is characteristic of algal cells that grow symbiotically in animal cells. This release can be stimulated in freshly isolated symbionts by the addition of extracts of host tissue. Early in this project we found that the cultured

symbionts from *Aiptasia pallida* showed no response to these host tissue extracts; indeed, release was often repressed. We established a population of the cultured symbionts growing in host tissue. Within two months the response of the algae was indistinguishable from that of the native symbionts, indicating that this release was a phenotypic response that depended on whether the symbionts were grown in culture or in symbiosis.

One explanation for the difference in carbon release by cultured versus symbiotic cells was that under the high nutrient conditions in culture, carbon release was repressed (i.e., the symbionts retained carbon for their own growth under nutrient-replete conditions.) We looked at carbon release by symbionts in hosts that were very well-fed, and in hosts that were starved for prolonged periods (up the three months). Indeed, carbon release by the algae in the anemones increased as starvation proceeded. One preliminary experiment with cultured algae indicated that release did increase slightly in cells that were nutrient-limited. We examined the nature of the photosynthetic products that were released by the algae. Typically glycerol is the major product that is released by these dinoflagellates. In short-term photosynthetic experiments with $^{14}CO_2$, we found no release of glycerol by the cultured algae; glycollic acid was the major product. Surprisingly, 14C experiments with the symbiotic algae from fed anemones also revealed little labeled glycerol; most of the released products were various organic acids. We did find some glycerol release by algae from starving anemones. In contrast, measurements of total glycerol showed substantial glycerol release by symbiotic algae, even from fed anemones. The results indicate that the glycerol that was released was largely pre-synthesized.

The second area of the HBOI work was to examine phosphatase activity in the cultured versus symbiotic algae. We chose these enzymes since they were easily assayed, and preliminary studies indicated differences in the pH profiles between the two states. The functions of these enzymes is to provide phosphorus by cleaving phospho-diester bonds. Surface phosphatase activity in algae is induced under phosphorus-limited conditions and is repressed when inorganic phosphate is present. Cultured symbionts grown at pH 8 exhibited little acid phosphatase activity, but had a broad pH peak between pH 7 and 9. In contrast, symbionts freshly isolated from the host showed a strong acid phosphatase peak at pH 5, and little activity at alkaline pH. When the cultured algae were maintained at pH 6 an acidic peak was induced, and when symbionts were isolated into normal pH 8 media, they exhibited an alkaline activity peak. Histochemical evidence indicates that the host cell vacuoles containing symbiotic zooxanthellae represent an acidic environment. Thus, the differences between the phosphatase activities of cultured

and symbiotic algae appear to be functions of the pH of the environment.

The third area of the HBOI work focused on the role of the symbiont cell surface in affecting cell recognition processes. The algae are intracellular symbionts, and the symbiosis is established through a suite of events involving initial cell-cell contact, phagocytosis of the potential symbiont by the host cell, and the proliferation of symbionts throughout host tissue. Using aposymbiotic (symbiont-free) *A. pallida*, we found that symbionts freshly isolated from symbiotic anemones were phagocytosed at high rates, whereas cultured cells were taken up only sparingly. Once in host tissue, both symbiotic and cultured cells proliferated at similar rates. We examined the uptake of the cultured cells with transmission electron microscopy, using facilities at the University of Georgia. These cells exist in two forms: non-motile cells with thickened cell coverings, and flagellated motile cells with a thin covering that is morphologically similar to that of symbionts growing in host cells. Motile cells in contact with the host cell surface were surrounded by a meshwork of microvilli that were not seen when non-motile cells contacted the surface. Apparently these motile cells have a cell surface chemistry that is similar to that of the normal symbionts.

One interesting sidelight of this work was a project done by Santiago Perez, one of my graduate students. He surveyed a variety of symbiotic dinoflagellates for infectivity in aposymbiotic *A. pallida*. In this study he developed a number of heterologous host / symbiont combinations. It is known that "coral bleaching" is a response to elevated temperatures in which symbionts are lost from host tissue. Perez found that these combinations "bleached" at different temperatures, depending on the sensitivity of the particular algal strain to elevated temperatures. Thus, the specificity of coral bleaching events appears to be a function of the symbiont, rather than of host tissue.

CONCLUSIONS: We have identified several areas where genetic regulation in the *Aiptasia pallida* symbiosis is likely to occur. The synthesis, storage and release of glycerol by the algal symbionts is a major area where "symbiotic genes" are likely to operate. A second major area is the response of the algal cell surface to the symbiotic and cultured states.

SIGNIFICANCE: These studies lay a foundation for understanding how genetic regulation occurs in this and other marine symbioses. Molecular techniques should be applied to the questions that we have studied to gain this understanding.

Publications supported by this grant:

Davy, S. K., and C. B. Cook 1999. The relationship between nutritional status and carbon flux in the zooxanthellate sea anemone *Aiptasia pallida* (Verrill). (Submitted to Marine Ecology Progress Series)

In preparation:

Annis, E. A., and C. B. Cook 1999. The effects of host feeding and phosphorus supply on phosphatase activity in the symbiotic dinoflagellate *Symbiodinium bermudense*. (To be submitted to Limnology and Oceanography)

Perez, S. F., C. B. Cook and W. R. Brooks 1999. The role of symbiotic dinoflagellates in the temperature-induced bleaching response of *Aiptasia pallida* (Anthozoa, Actinaria). (To be submitted to the Journal of Experimental Marine Biology and Ecology).

Theses supported by this grant:

Annis, E.A. 1998. Phosphatase Activity as an Indicator of Phosphorus Sufficiency in the Symbiotic Dinoflagellate *Symbiodinium bermudense*. (M.S. Thesis, Florida Institute of Technology, May 1998)

Hrdlicka. L. A. 1999. The Effects of Symbiosis on Glycerol Metabolism in the Symbiotic Sea Anemone *Aiptasia pallida* (M. S. Expected August 1999)